amined. Amylopectin in 1.25% solutions was treated with six equivalents of potassium chlorate at *p*H 3, 5 and 7. No oxidant was consumed in 5 days at room temperature. Even amylopectin oxidized by hypochlorite at *p*H 7 was not oxidized by chlorate when the *p*H was reduced to 3.

Carbon Dioxide Evolved.—Carbon dioxide produced during oxidation of amylopectin with hypochlorite was determined for reactions at *p*H 3, 5, 7 and 9. For each determination 5 g. of amylopectin was dissolved in 200 ml. of hot water, 92.5 ml. of 2 *N* sodium hypochlorite solution (3 moles/mole D-glucose unit) was added and the solution was made up to 450 ml. Concentrated sodium hydroxide solution or hydrochloric acid was added as necessary to produce the desired *p*H values. After the oxidant was consumed each solution was acidified with about 4 ml. of concentrated sulfuric acid in 20 ml. of water and the mixture refluxed in a contained system swept by a slow stream of nitrogen. Carbon dioxide evolved was measured by passage into a 0.5 *N* solution of sodium hydroxide. Differential titration of the alkali with 0.1 *N* oxalic acid, after addition of barium chloride solution in excess of the carbonate present, gave a measure of the carbon dioxide absorbed.

Since in oxidations at *p*H 3 and 5 the produced carbon dioxide is immediately evolved, the oxidation was performed in a flask swept by nitrogen. The gas stream was passed into sodium hydroxide as before, but chlorine as well as carbon dioxide was absorbed. Thus the amount of alkali neutralized by chlorine was also determined by treating the solution, titrated to the neutral point, with iodide and concentrated hydrochloric acid and titrating of iodine with thiosulfate. Carbon dioxide produced by the oxidation at *p*H 3 and 5 is shown in Table II.

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LAFAYETTE, INDIANA

[CONTRIBUTION NO. 26 FROM THE OLYMPIC RESEARCH DIVISION, RAYONIER INC.]

Graded Acid Hydrolysis Studies of a Xylan Polyuronide Associated with Wood Cellulose from Western Hemlock[1]

BY J. KELVIN HAMILTON AND NORMAN S. THOMPSON

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A series of oligosaccharides and oligouronides has been obtained by the graded acid hydrolysis of a xylan polyuronide associated with a wood cellulose from western hemlock. At least six distinct acidic substances composed of 4-O-methyl-D-glucuronic acid and D-xylose and four reducing oligosaccharides composed of D-xylose were detected by suitable chromatographic techniques. Three of the oligosaccharides showed the same mobility as authentic specimens of xylobiose, xylotriose and xylotetraose on all chromatographic solvents that were tried. The acidic substances were 4-O-methyl-D-glucuronic acid, 2-O-(4-O-methyl-α-D-glucuronopyranosyl)-D-xylose and an aldotriouronic acid which crystallized as a trihydrate. This acid has been identified by methylation studies as O-α-4-O-methyl-D-glucuronopyranosyl-(1 → 2)-O-β-D-xylopyranosyl-(1 → 4)-D-xylopyranose. A tentative structure of the parent xylan polyuronide based upon the oligosaccharides and oligouronides that have been identified is described.

The hemicelluloses which remain associated with wood cellulose produced from western hemlock by the sulfite process have been shown to be a mixture of two families of polysaccharides, namely, glucomannans and xylan polyuronides.[2] The predominant polysaccharides were found to be the glucomannans in which the ratio of D-glucose to D-mannose was 1 to 3.[3] The xylan polyuronides varied in their ratio of 4-O-methyl-D-glucuronic acid to D-xylose from 1 to 4 to 1 to 8. Controlled periodate oxidation studies were carried out on these two types of hemicelluloses. These data, in conjunction with specific rotations of the polysaccharides before and after hydrolysis, indicated that both of the above hemicellulose series were linked predominantly by 1 → 4-β-glycosidic bonds. Prolonged periodate oxidation, followed by reduction and hydrolysis, also indicated that the glucomannans were predominantly straight chain and the xylan polyuronides were slightly branched. Methylation and graded hydrolysis studies on the glucomannans are in agreement with these results.[3] Recent studies have shown that the xylan polyuronides can be easily separated from the glucomannans by suitable extraction procedures.[4] The xylan polyuronides used in these experiments were isolated by a modification of one of these procedures (see Experimental).

Information concerning the sequence of the sugar residues in these xylose-containing hemicelluloses may be obtained by stepwise degradation and characterization of oligosaccharides or oligouronides of varying degree of polymerization. This paper is concerned with the partial degradation of the xylan polyuronide components of this wood cellulose system by graded acid hydrolysis and the isolation, identification and characterization of certain members of the oligouronides so obtained.

In many instances acid-containing polysaccharides have been hydrolyzed and found to give large amounts of aldobiouronic and aldotriouronic acids. In only a few instances has the monomeric uronic acid been isolated in good yields under normal conditions of hydrolysis.[5] The studies of Haworth,[6] Anderson[7] and O'Dwyer[8] on plant gums and hemicelluloses from various plant sources are classics in this field. Anderson and Otis[9] in 1930 reported the isolation of a methylhexuronic acid from mesquite gum. Later, Anderson[10] and Sands[11,12]

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in their studies on the hemicelluloses associated with cottonwood and mesquite wood, respectively, reported the isolation of a series of oligouronides composed of this methylhexuronic acid and D-xylose and extending from the parent acid to the aldotriouronic acid. In 1948, White,[13] in a detailed study on mesquite gum, identified the methylhexuronic acid as 4-O-methyl-D-glucuronic acid. This subsequently was confirmed in greater detail by Smith.[14] Recent studies indicate that this acid is very widely distributed in plant materials.[15-22]

In this Laboratory by the employment of various graded hydrolysis techniques, it has been possible to obtain a series of oligouronides comprised of 4-O-methyl-D-glucuronic acid and D-xylose and extending from the parent monomeric acid to what is believed to be an aldoheptaouronic acid as well as a series of neutral oligosaccharides. These latter substances were chromatographically identical with authentic specimens of xylobiose, xylotriose and xylotetraose. Reduction of the isolated oligouronides with sodium borohydride followed by very mild hydrolysis produced the next chromatographically fastest moving reducing oligouronide indicating that these acids belonged to a closely related series. The fact that these oligouronides exhibited a decreasing optical rotation with increasing degree of polymerization indicated that the anhydroxylose residues were joined to one another by β-glycosidic bonds.

The 4-O-methyl-D-glucuronic acid was characterized as the α-form of the amide derivative of the methyl glucuronoside and the D-xylose as its dibenzylidene dimethyl acetal derivative.[2] The aldobiouronic acid proved to be 2-O-(4-O-methyl-α-D-glucuronopyranosyl)-D-xylose by its equivalent weight, optical rotation and the sugars obtained following methylation, reduction with lithium aluminum hydride, methylation and hydrolysis. This acid has previously been detected in the holocellulose of western hemlock.[19,23] A crystalline hydrated aldotriouronic acid was obtained and was subsequently shown to be O-α-4-O-methyl-D-glucuronopyranosyl-(1→2)-O-β-D-xylopyranosyl-(1→4)-D-xylopyranose (I) (see Experimental). Various paper partition chromatographic techniques indicated that some of the remaining acids might be the aldotetra-, aldopenta-, aldohexa- and aldoheptaouronic acids. Paper electrophoresis suggested that the supposed aldotetra- and aldopentaouronic acids were probably isomeric mixtures of aldotetra- and aldopentaouronic acids, respectively.

Recently an aldotriouronic acid has been reported from a hydrolyzate of a xylan polyuronide from aspenwood.[24] The structure of this crystalline non-hydrated acid has not been reported but may be very similar to the one discussed in this paper. An aldotriouronic acid containing 4-O-methyl-D-glucuronic acid and xylose has also been reported from Monterey pine.[25]

In the case of the crystalline hydrate of the aldotriouronic acid obtained in this study, the point of attachment of the 4-O-methyl-D-glucuronic acid and D-xylose residues to one another was determined from a study of the fully methylated trisaccharide II, obtained by reduction with lithium aluminum hydride and methylation of the aldotriouronic methyl ester.

Hydrolysis of II and chromatographic separation of the components yielded 2,3-di-O-methyl-D-xylose, 3,4-di-O-methyl-D-xylose and 2,3,4,6-tetra-O-methyl-D-glucose. It was found that paper electrophoresis using a borate buffer at *p*H 9.2 also could be employed for the separation of 3,4-di-O-methyl-D-xylose from the other two hydrolytic products. The 2,3,4,6-tetra-O-methyl-D-glucose crystallized and was characterized by its melting point and specific rotation. The 2,3-di-O-methyl-D-xylose was characterized as its crystalline anilide. The 3,4-di-O-methyl-D-xylose was oxidized and characterized as its crystalline δ-lactone.

It was noted previously[2] that these polyuronides appeared to be hydrolyzed more readily to their monomeric constituents than other similar polymers, at least when compared with the conditions quoted in the literature.[5,21,23,26-28] A study was made of the ease of hydrolysis of the aldobiouronic acid from this xylan polyuronide and with aldobiouronic acids from cherry gum.[29] One of these acids was probably 2-O-β-D-glucuronopyranosyl-D-mannose described earlier by Jones,[30] while the other was a new acid from this source which, qualitatively, was composed of equal amounts of galactose and glucuronic acid. When compared under identical conditions such as concentration of carbohydrate, acid strength, time and temperature, the aldobiouronic acid from the polyuronide was hydrolyzed with greater facility than the other two.

Based on the presently accepted evidence for the structure of xylan polyuronides, the presence of isomeric oligouronides above the aldobiouronic acid is to be expected. A chromatographic search was carried out by paper chromatography and paper electrophoresis with particular emphasis on the mother liquors from the crystalline hydrated aldotriouronic acid in order to obtain, if possible, an isomer of this compound. No such isomeric acid was observed. However, a series of experiments, which will be reported later, has shown that this acid travels at the same chromatographic rate as the crystalline aldotriouronic acid reported here

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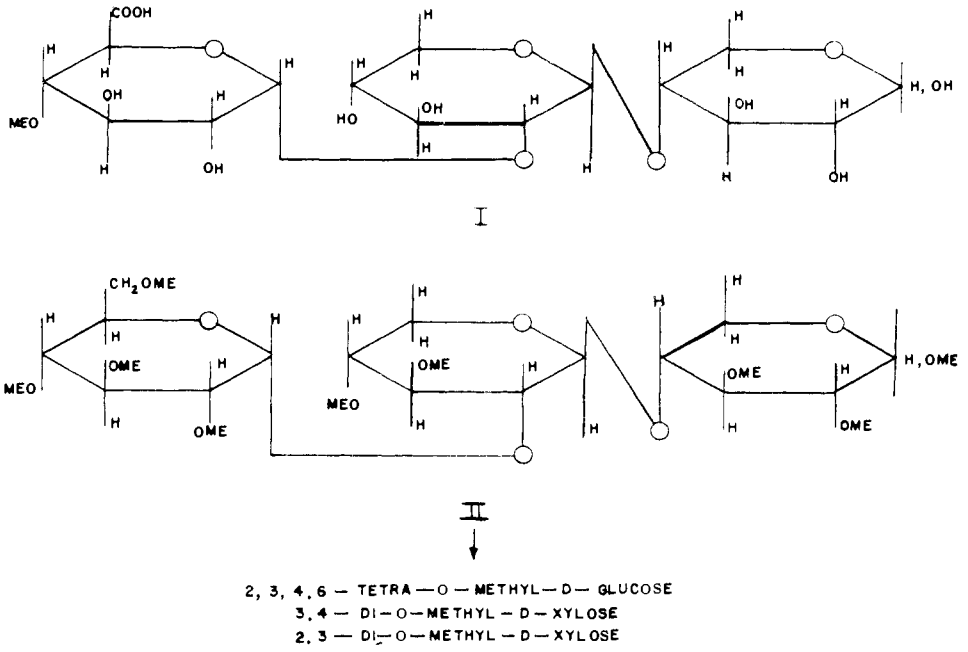
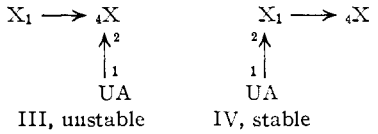


Fig. 1.

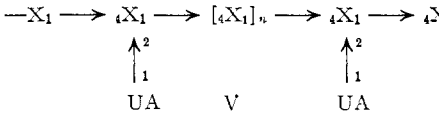
and that therefore the isomer might have been present in small quantities in the mother liquors.

The absence of large amounts of an isomeric aldotriouronic acid may possibly be explained by the facility with which the stabilizing effect of a uronic acid-type carboxyl group is transmitted to those glycosidic bonds which are further removed from the uronic acid moiety.



X = D-xylose; UA = 4-O-methyl-D-glucuronic acid

The structure of the xylan polyuronides from which these oligosaccharides and oligouronides were obtained appears to fit into a pattern that is general for other similar polymers from various plant materials.[15-22] The backbone of the polymer is a chain of D-xylopyranose residues joined predominantly by 1→4-β-glycosidic bonds. At frequent intervals a 4-O-methyl-D-glucuronic acid residue is attached to position 2 of a xylose moiety of the main chain. These uronic acid groups occur on an average of one to every 4 to 8 xylose residues.



n = 2 to 6
X = D-xylose
UA = 4-O-methyl-D-glucuronic acid

It has been reported recently that an araboxylan polyuronide is present in holocellulose from western hemlock.[18] Preliminary results indicate that similar polymers are present in holocelluloses from other conifers.[19] These arabinose residues are readily removed during the sulfite process, leaving a family of xylan polyuronides of the above structure in which the ratio of acid groups to xylose and also the chain length as determined viscometrically vary considerably Xylan polyuronides from other wood celluloses produced by the sulfite process also conform to the above pattern.[29]

A more complete picture of the structure of these xylan polyuronides will have to await the isolation and characterization of the methylated residues obtained from the hydrolysis of the fully methylated polysaccharide. This work is now in progress.

Experimental

Paper Chromatography.—Chromatographic solvents employed for the separation of uronic acids, sugars and low molecular weight oligosaccharides were (A) ethyl acetate–acetic acid–formic acid–water (18:3:1:4) ethyl acetate–acetic acid–water in various ratios of (9:2:2) (B), (3:1:3) (C) and (6:3:2) (D). Solvents for the separation of neutral sugars and oligosaccharides from acid-containing fragments were ethyl acetate–pyridine–water in ratios of (8:2:1) (E) and (2:1:2) (F) and butanol–pyridine–water (10:3:3) (G). The chromatographic solvents employed to separate oligosaccharides (arranged in increasing rapidity of movement, larger oligosaccharides separated and decreasing resolution of substances of similar degree of polymerization) were ethyl acetate–acetic acid–water in ratios of (6:3:2) (D), (6:3:3) (H) and (6:3:4) (I).

Solvents for the separation of methylated sugars were benzene–ethanol–water (169:47:15) (J) and (196:66:8) (K), benzene–ethanol–water–formic acid (169:47:15:2) (L) and methyl ethyl ketone–water azeotrope (M). There exists a range of benzene–ethanol–water solvents from (239:65:15) to (303:38:3) which will separate 2,3- from 3,4-di-O-methylxylose with varying degrees of efficiency. The addition of a small amount of formic acid to the solvents sharpened the resolution.

The indicators employed were ammoniacal silver nitrate for non-reducing substances, brom cresol blue buffered to pH 6 for carboxylic acids and p-anisidine–trichloroacetic acid. A modification of the last spray reagent to which 4% by volume of pyridine had been added was used to detect pentoses and pentose oligosaccharides. This reagent gave chocolate brown spots (intensified under ultraviolet light) with all four pentoses, oligosaccharides composed of xylose, oligouronides containing more than one xylose residue and oligosaccharides of arabinose. Hexoses gave a yellow-brown spot

and tetroses a somewhat darker yellow-brown. Hexose–pentose disaccharides gave an intermediate color.[31]

Chromatographic papers employed were Whatman No. 1, No. 3 and occasionally No. 50.

The Isolation of Hemicelluloses.—The wood cellulose employed for this study contained 89.4% α-, 2.3% β- and 8.3% γ-cellulose and was produced from western hemlock (*Tsuga heterophylla*) by the sulfite process.

Twenty-two kilograms of the wood cellulose was extracted in three equal batches with 18% NaOH. After 20 minutes, the batch was pressed to twice its original weight and the hemicellulose-containing caustic was drained into large stainless steel containers. The liquors from the three batches were combined (104 liters), acidified with acetic acid (with cooling) and allowed to stand until a precipitate had settled (130 g.).

The liquors were siphoned from the precipitate, were dialyzed for 8 days against running water and concentrated to one-tenth of the original volume. The thick magma resulting from the evaporation of the liquors was precipitated by 3 volumes of methanol. The precipitate was isolated on the centrifuge, combined with the acid-insoluble fraction and dried by solvent exchange through methanol and ether. The mixture, weighing 1472 g., was called "Hemicellulose Mixture A."

This hemicellulose and the others reported in this paper were analyzed by the techniques described in a previous communication.[2] Inasmuch as glucose, mannose, xylose and 4-O-methyl-D-glucuronic acid previously were shown to be present in this wood cellulose, no further characterization was carried out.[2]

Found for Hemicellulose Fraction A: molar ratio of glucose, mannose, xylose and 4-O-methyl-D-glucuronic acid after hydrolysis, 1:1.9:1.2:0.1; % ash, 7.0; % moisture, 10.9; $[\alpha]^{25}D$ −48° (*c* 1, 8% NaOH).

The Isolation of a Crude Xylan Polyuronide.—Hemicellulose Mixture A (1.2 kg.) was extracted with 15 liters of 0.1 *N* sodium hydroxide for two days. The insoluble fraction was recovered on the centrifuge and washed successively with 10 liters of water and 11 liters of 0.1 *N* acetic acid. The residue was isolated by centrifugation, dialyzed for one week, solvent exchanged and finally air-dried. This product, called "Hemicellulose Fraction C," weighed 600 g. and was predominantly a glucomannan.

The alkaline supernatant and washings were deionized with an excess of Amberlite IR 120 (H) resin. The resin was removed by filtration and the somewhat turbid acidic liquors were flooded with 3 volumes of acetone. After five days, the precipitate was isolated by centrifugation, solvent exchanged through acetone and ether and air-dried. This fraction (380 g.) was designated "Hemicellulose Fraction B."

Found: Molar ratio of glucose:mannose:xylose:4-O-methyl-D-glucuronic acid, 1:2:8:1; equivalent weight per carboxyl, 1900; equivalent weight per methoxyl, 1840; $[\alpha]^{25}D$ −54.3° (*c* 1, water), $[\alpha]^{25}D$ −66.8° (*c* 1, in 10% KOH); intrinsic viscosity in cupriethylenediamine hydroxide (cuene), 0.27; % ash, 1.9; % moisture, 15.3.

Only 10 g. of carbohydrate material remained soluble in the acetone–water medium. It is interesting to note that this small fraction contained a mixture of soluble polymers which, upon hydrolysis and chromatographic separation with solvents C and G, gave substances identical with glucose, mannose, xylose, 4-O-methyl-D-glucuronic acid and galactose.

Preliminary Experiments.—A number of preliminary experiments were carried out in an attempt to discover satisfactory conditions for graded hydrolysis. Hydrolysis was carried out under reflux employing 0.05 *N* sulfuric acid, 0.1 *N* sulfuric acid and 0.25 *N* sulfuric acid, samples being removed each hour for 8 hr. A six-month hydrolysis in *N* sulfuric acid at room temperature also was investigated.

The hydrolyzates were worked up according to the procedure described below and were separated into neutral and acidic constituents. Xylose and xylose-containing oligosaccharides were the first substances detected chromatographically. After 5 hr. with 0.05 *N* sulfuric acid, 2 hr. with 0.1 *N* sulfuric acid and 1 hr. with 0.25 *N* sulfuric acid, appreciable quantities of 4-O-methyl-D-glucuronic acid were detected as well as glucose, mannose and several hexose-containing oligosaccharides.

(31) A. Beélik and J. K. Hamilton, unpublished results.

Appreciable amounts of short chain polymers were also present and were collected into two separate fractions, one composed of oligouronides and the other of oligosaccharides. The constituents of these fractions were separated from one another by paper partition chromatography using the appropriate solvent system. The individual sugars were removed by elution of the appropriate zones with water.

The neutral fraction contained xylose, mannose, glucose and several oligosaccharides which gave xylose only upon hydrolysis for 8 hr. under reflux in *N* sulfuric acid. These latter substances travelled at the same rate with solvents A B and E as did authentic specimens of xylobiose, xylotriose and xylotetraose. Some oligosaccharides containing only hexose units were detected also.

The acidic fraction contained 4-O-methyl-D-glucuronic acid and six additional spots thought to range from the aldobiouronic acid to the aldoheptaouronic acid. These substances could be resolved in 24 to 36 hr. with solvent I. Complete hydrolysis of each of these acids by refluxing in 1 *N* sulfuric acid for 10 hr. gave substances chromatographically identical to 4-O-methyl-D-glucuronic acid, xylose and slight traces of aldobiouronic acid with solvents A, B and C.

It is interesting to note that a plot of the logarithm of the distance the oligouronide traveled *versus* the order of appearance on a paper chromatogram gave essentially a straight line. This was taken as further evidence in favor of a homologous series of oligouronides (see Fig. 2).

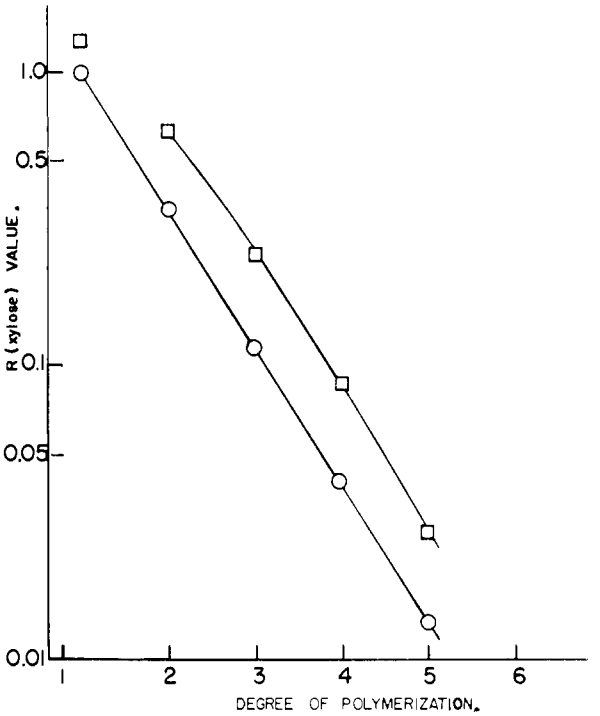


Fig. 2.—Chromatographic behavior of oligouronides and oligosaccharides. The solvent system employed was ethyl acetate:acetic acid:water, 3:1:3 vol./vol. (upper phase). The time was 96 hours: □, xylose and 4-O-methyl-D-glucuronic acid produced by acid hydrolysis; O, xylose only produced by acid hydrolysis.

Each of the acids up to the aldopentaouronic acid were subjected to paper electrophoresis.[32] The low D.P. acids up to the aldotriouronic acid could not be resolved into more than one spot while the aldotetraouronic and aldopentaouronic acids gave a number of overlapping spots when sprayed with *p*-anisidine indicator.

The ease of hydrolysis of the aldobiouronic acid from the xylan polyuronide was compared with those of the hexose–

(32) Electrophoresis experiments were carried out on a Karler–Misco Electrochromatographic Apparatus supplied by the Microchemical Specialties Co., Berkeley, Calif., using tenth molar sodium borate at *p*H 9.1 and 700 volts at 11 to 35 milliamp. for 6 hr.

hexuronic acids from cherry gum. The oligouronides from cherry gum were prepared by hydrolyzing 15 g. of gum (purified by methanol precipitation) with 1 l. of *N* sulfuric acid for 8 hr. The two acids were isolated in about 1.5% yield each by the techniques described below. Hydrolysis of these two acids in 2 *N* sulfuric acid for 8 hr. under reflux gave equal amounts of glucuronic acid and mannose for one acid and equal amounts of glucuronic acid and galactose for the other acid, as indicated by qualitative paper chromatography.

Fifty milligrams each of the aldobiouronic acid (xylan polyuronide) and the two cherry gum uronic acids were hydrolyzed separately for 10 hr. with 50 ml. of *N* sulfuric acid under reflux. After the usual isolation procedure, chromatography of the hydrolyzates indicated qualitatively that the two cherry gum uronic acids were more difficult to hydrolyze than the aldobiouronic acid derived from the xylan polyuronide.

Reduction Experiments.—A small quantity of the second acidic substance (aldobiouronic acid) (50 mg.), obtained from the graded acid hydrolysis of the xylan polyuronide, was reduced with sodium borohydride. After removal of sodium ion on Amberlite IR 120 (H) and borates by distillation with methanol, the non-reducing carbohydrate was hydrolyzed for 8 hr. with *N* sulfuric acid. Only 4-O-methyl-D-glucuronic acid could be detected by chromatography indicating that this acidic substance was an aldobiouronic acid.

A similar reduction was carried out on the third (or aldotriouronic) acid. Hydrolysis for 8 hr. under reflux with 0.5 *N* sulfuric acid produced 4-O-methyl-D-glucuronic acid, xylose, xylitol and the aldobiouronic acid mentioned above.

This reduction technique was repeated on the aldotriouronic acid as well as on the aldotetra-, aldopenta-, aldohexa- and aldoheptaouronic acids. After a 15-minute hydrolysis with *N* sulfuric acid in a boiling water-bath, each acid produced the next lower of the series when examined by paper chromatography. The aldotriouronic acid gave the aldobiouronic acid exclusively; the aldotetraouronic acid gave aldotriouronic acid; the aldopentaouronic acid gave aldotetra- and aldotriouronic acids; the aldohexaouronic acid gave aldopenta-, aldotetra- and aldotriouronic acids; while the aldoheptaouronic acid gave aldohexa-, aldopenta-, aldotetra- and aldotriouronic acids. Xylose, in certain cases, was the only neutral reducing sugar detected.

Graded Hydrolysis.—Eighty grams of Hemicellulose Fraction B was shaken in 20 g. batches with 1160 ml. of distilled water. After heating under reflux for 1 hr., the hemicellulose had dissolved almost completely, and 166 ml. of 2 *N* sulfuric acid was added. The reaction was allowed to proceed under gentle reflux for 3 hr. After cooling in cold running water, barium carbonate was added to the hydrolyzate, and the *p*H was decreased to 6 with acetic acid after removal of excess barium carbonate. The solution was evaporated to a thick sirup, and 500 ml. of methanol and several grams of barium carbonate were added. An equal volume of ethanol was added with vigorous shaking. A voluminous white precipitate was allowed to settle over several days and was finally recovered by centrifugation. The yellow, alcoholic solution was found to contain xylose, mannose, glucose, xylobiose, xylotriose and two hexose disaccharides when examined chromatographically.

The barium salts were dissolved as completely as possible in 100 ml. of water, and an excess of Amberlite IR 120(H) was added. After shaking for 48 hr., the resin was removed and Duolite A-4 ion-exchange resin and a steel screen capsule containing Amberlite IR 120(H) ion-exchange resin were added. After shaking for 48 hr., the mixture was filtered and the resin washed twice with 100-ml. portions of water. The filtrate and washings contained xylose and neutral oligosaccharides. In those instances where oligouronides were detected, the concentrates were diluted with water and the acids extracted as before.

The Duolite A-4 ion-exchange resin was shaken for 24 hr. with 200 ml. of *N* sulfuric acid, filtered and washed with water until the washings were neutral. The filtrate and washings were neutralized with barium carbonate, filtered, freed of barium ion with Amberlite IR 120(H) ion-exchange resin and evaporated to a sirup.

Separation of Acidic Components.—The four sirups were combined (10 g.) and examined qualitatively by paper chromatography on solvents A, B, E and D. Strong spots corresponding to 4-O-methyl-D-glucuronic acid, an aldobiouronic acid and an aldotriouronic acid were detected while smaller spots corresponding to xylose, an aldotetraouronic acid and an aldopentaouronic acid also were present.

The remaining sirup was spotted on bands 12 cm. long (containing 200 mg. of sugars) on sheets of Whatman No. 3 chromatographic paper measuring 19 × 54 cm. Solvent Mixture D was chosen as satisfactory because of the low D.P. of the oligouronides present and because it could resolve 4-O-methyl-D-glucuronic acid from xylose satisfactorily. The chromatograms ran overnight, after which time 4-O-methyl-D-glucuronic acid was at the bottom of the sheet, and the aldopentaouronic acid was 3 cm. from the starting line. The portion of the paper corresponding to each acid was cut out, combined with others and removed from the paper with water. The dark sirups obtained after evaporation of the aqueous solutions were triturated with 50% aqueous methanol and evaporated to yellow sirups. The results of this study are shown in Table I.

TABLE I

OLIGOURONIDES OBTAINED BY GRADED ACID HYDROLYSIS[a]

Compound	$[\alpha]^{25}D$	Equiv. wt. Found	Equiv. wt. Calcd.	Yield, %
A[c]	+85°	200	208	0.75
AX	+95	364	340	3.2
AX_2[b]	+62	520	526	2.1
AX_3	+25	..	604	0.4
AX_4	+ 7	..	736	0.1

[a] 0.25 *N* H_2SO_4, 3 hr., 100°. [b] Contains $3H_2O$ of crystallization; $[\alpha]^{25}D$ calculated on anhydrous basis. [c] A = 4-O-Methyl-D-glucuronic acid, X = D-xylose.

Characterization of the Aldobiouronic Acid.—The second acidic substance, which by qualitative tests behaved as an aldobiouronic acid, was further purified *via* the barium salt and precipitation with an excess of methanol. After removal of the barium salts with Amberlite IR 120(H) resin, the acid was isolated as a thick, slightly yellow sirup.

Anal. Calcd. for 2-O-(4-O-methyl-α-D-glucuronopyranosyl)-D-xylose: equiv. wt., 340; $[\alpha]^{25}D$ ±95°.[21] Found: equiv. wt., 364; $[\alpha]^{25}D$ +95°.

The methyl ester, methyl glycoside of the aldobiouronic acid was methylated, reduced and methylated by the procedure of Abdel-Akher and Smith.[33] Hydrolysis, then paper chromatography on solvents J, K and L showed the presence of 2,3,4,6-tetra-O-methylglucose and 3,4-di-O-methylxylose.

The Isolation of Crystalline Aldotriouronic Acid.—A portion of the third acidic substance (the aldotriouronic acid), which had been used for chromatographic purposes, crystallized in its container. The remaining sirupy acid (3.5 g.) was therefore dissolved in 50% aqueous methanol, centrifuged and evaporated to a thin sirup which, upon nucleation, gave masses of crystals imbedded in a dark brown, sirupy matrix. The mixture was placed on a piece of clean, porous plate, and after 24 hr. the dry, white crystals were scraped off with a spatula. The porous plate was crushed to a powder and extracted with four 100-ml. portions of boiling water. The extract was evaporated to a mass of crystals and sirup, and the process was repeated. After the fourth extraction, the sirupy mass (1 g.) crystallized (even after nucleation) only after standing several months.

Since tests showed the partially purified acid would now crystallize readily from water, the remainder (2.2 g.) was dissolved in 50 ml. of water and evaporated at 40° under diminished pressure until a convenient amount had crystallized (0.4 g.). This crop was isolated by centrifugation, was washed twice with water and dried *in vacuo* over phosphorus pentoxide. The remaining acid (1.8 g.) was employed for methylation and other studies. The purified crystals had no melting point but gave off water (turned copper sulfate blue) above 100°. Slow heating gave an uncertain melting point of 184°. The crystals lost no water when stored at 0.1 mm. pressure at 60° for 2 hr. Water was lost when the crystals were heated at 110° for 3 hr.

Anal. Calcd. for $C_{16}H_{26}O_{14}(OCH_3)\cdot 3H_2O$: equiv. wt., 526; OCH_3, 5.89; C, 38.8; H, 6.46; H_2O, 10.3. Found: equiv. wt., 520; OCH_3, 5.73; C, 38.8; H, 6.46; H_2O, 11.0; $[\alpha]^{25}D$ +57° (*c* 1, water).

Methylation of the Aldotriouronic Acid.—One gram of the once-purified crystals of the aldotriouronic acid was dis-

(33) M. Abdel-Akher and F. Smith, *Nature*, **166**, 1037 (1950).

solved in 50 ml. of 1% methanolic hydrogen chloride and allowed to stand at room temperature for one week. Periodically, aliquots were analyzed by chromatography and suitable indicators on solvent D. This technique showed the gradual disappearance of the aldotriouronic acid (R_x 0.35), the appearance and disappearance of the acidic methyl glycoside (R_x 0.46) and the complete formation of the non-reducing methyl ester, methyl glycoside (R_x 0.86).

The ester-glycoside (0.943 g.) was isolated by evaporation of excess solvent after the removal of chloride ion with silver carbonate. It was dissolved in a mixture of 10 ml. of anhydrous methanol and 20 ml. of methyl iodide and cooled to 0°. One gram each of silver oxide and Drierite were added, and the mixture, after remaining at 0° for 4 hr., was maintained at room temperature for an additional 18 hr. The mixture was then heated under reflux for 1 hr., and the product was isolated (0.941 g.). Another methylation, employing methanol as solvent, was accomplished under reflux for 8 hr. with fresh reagents. Five additional methylations with acetone as solvent had to be carried out before the product was soluble in methyl iodide only. Three additional Purdie methylations gave 1.1 g. of ether-soluble oil.

The methylated aldotriouronic acid, in 50 ml. of anhydrous ether, was added dropwise to 1.1 g. of lithium aluminum hydride in an equal volume of ether. The mixture was refluxed gently for 1 hr., allowed to stand overnight and refluxed for an additional 0.5 hr. The excess lithium aluminum hydride was then destroyed with a small amount of ethereal ethyl acetate.

The organic mixture was shaken with 500 ml. of water and the mixture was evaporated free of organic solvent. The aqueous suspension was filtered, the washings combined with the filtrate, and the clear, aqueous solution was neutralized by shaking with an excess of Amberlite IR 120(H) and Duolite A-4 ion-exchange resins. The cloudy sirup obtained after evaporation was triturated with ether to give, after evaporation, 0.773 g. of a clear, colorless sirup.

The partly methylated trisaccharide was methylated three times with Purdie reagents. The color developed in this reaction was removed from an aqueous solution with mixed acid–base resins. Evaporation gave a thick, colorless sirup weighing 0.63 g.

Anal. Calcd.: OCH_3, 48.9. Found: OCH_3, 46.6; $[\alpha]^{25}D$ +49° (*c* 0.64, water).

Hydrolysis of the Methylated Trisaccharide and Identification of Components.—The methylated trisaccharide (0.47 g.) was dissolved in 140 ml. of *N* sulfuric acid and heated under gentle reflux for 12 hr. The hydrolyzate was worked up using barium carbonate and mixed acid–base resins. Evaporation gave a light yellow sirup (0.437 g.) which, upon chromatography in solvents J, K and L, revealed three major spots corresponding in mobility and color reactions to authentic 2,3-di-O-methylxylose (R_g 0.48), 3,4-di-O-methylxylose (R_g 0.55) and 2,3,4,6-tetra-O-methylglucose (R_g 1.0) (solvent J). A very slight trace of a slow-moving spot (R_g 0.1) was also detected.

The sirupy mixture of methylated sugars were separated on solvent L. The chromatograms were run for 48 hr., and the tetramethylglucose which ran off the end of the paper was collected in shallow glass dishes in the bottom of the chromatographic jars. The tetramethylglucose was separated from a large amount of colored debris by chromatography for 6 hr. on solvent M. The sections of the paper corresponding to the sugars were cut out, extracted with water, filtered, deionized with mixed acid–base resin and triturated with chloroform. Evaporation gave 70 mg. of 2,3-di-O-methylxylose, 72 mg. of 3,4-di-O-methylxylose and 110 mg. of 2,3,4,6-tetra-O-methylglucose which crystallized spontaneously.

The crystalline tetramethylglucose was recrystallized from ether and petroleum ether. The first 20 mg. was collected for analysis and melted at 89–90° and showed $[\alpha]^{25}D$ +83° (*c* 1, water) constant value.

Twenty milligrams of 2,3-di-O-methylxylose was employed for the preparation of the characteristic anilide derivative.[34] The m.p. and mixed m.p. was 125–126°. Two crystalline structures exist for the anilide of 2,3-di-O-methylxylose melting at 125 and 145°, respectively.[35]

The lactone derivative of 3,4-di-O-methylxylose was prepared using 28 mg. of sirup by standard techniques.[36] The 3,4-di-O-methyl-D-xylono-δ-lactone melted at 65–66° and showed $[\alpha]^{25}D$ −19.4° (*c* 1.2, water).

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

Reduction of the Products of Periodate Oxidation of Carbohydrates. V. The Constitution of Cellulose[1]

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Cellulose has been subjected to prolonged oxidation with sodium periodate and with periodic acid. The polyaldehyde so formed was reduced with sodium borohydride to the corresponding polyalcohol. Hydrolysis of the latter gave erythritol, glycolic aldehyde and also small amounts of glycerol and glucose (0.1–0.2%). After retreatment of the cellulose polyalcohol with periodate the product still contained glucose residues. The structural significance of these findings is discussed.

In previous communications[2–4] a new method has been reported for the determination of the fine structure of polysaccharides. This general method based upon periodate oxidation, followed by reduction and hydrolysis, has been extended in order to gain further insight into the detailed structure of cellulose, the most abundant of all naturally occurring organic substances.

It is generally accepted that cellulose is a long chain polysaccharide in which the anhydroglucose residues are joined predominantly by 1,4-β-glycosidic bonds.[5,6] Most investigators assume

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